

Fig. 1

Mutant-specific oligonucleotide primers used for mutant number 1. Mutated nucleotide underlined

	10	20	30	40	50
	—	—	—	—	—
<i>Bet v 1</i> sense	5'-	AATTATGAGACTGAGACC	<u>ACCT</u>	TCTGTTATCCCAGCAGCTCG	-3'
<i>Bet v 1</i> non-sense	3'-	TTAATACTCTGACTCTGGT	<u>GG</u>	GAGACAATAGGGTCGTCGAGC	-5'
sense primer	5'-		TGAGACCC	CTCTGTTATCCCAG	-3'
non-sense primer	3'-	ATACTCTGACTCTGGG	<u>GG</u>	GAGACA	-5'

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Fig. 2

Oligonucleotide primers for site directed mutagenesis of
Bet v 1 (No. 2801).

all	sense	1: 183Bv, 15-mer	5'-GTTGCCAACGATCAG
1	sense	2: 184Bv, 23-mer	5'-TGAGACCCCTCTGTTATCCCAG
1	non-sense	3: 185Bv, 23-mer	5'-ACAGAGGGGGTCTCAGTCTCATA
2	sense	4: 186Bv, 31-mer	5'-GATACCTCTTTCCACAGGTTCACCCCAAG
2	non-sense	5: 187Bv, 31-mer	5'-ACCTGTGGAAAGAGGGTATCGCCATCAAGGA
3	sense	6: 188Bv, 23-mer	5'-AACATTTAGGAAATGGAGGGCC
3	non-sense	7: 189Bv, 23-mer	5'-TTTCCTGAAATGTTTTCAACACT
4	sense	8: 190Bv, 23-mer	5'-TTAAGAACATCAGCTTTCCCGAA
4	non-sense	9: 191Bv, 23-mer	5'-AGCTGATGTTCTTAATGGTTCCA
5	sense	10: 192Bv, 23-mer	5'-GGACCATGCAAACCTCAAATACA
5	non-sense	11: 193Bv, 23-mer	5'-AGTTTGATGGTCCACCTCATCA
6	sense	12: 194Bv, 23-mer	5'-TTTCCCTCAGGCCTCCCTTTCAA
6	non-sense	13: 195Bv, 23-mer	5'-AGGCCTGAGGGAAAGCTGATGTT
7	sense	14: 196Bv, 24-mer	5'-TGAAGGATCTGGAGGGCCTGGAAC
7	non-sense	15: 197Bv, 24-mer	5'-CCCTCCAGATCCTTCAATGTTTTC
8	sense	16: 198Bv, 24-mer	5'-GGCAACTGGTGATGGAGGATCCAT
8	non-sense	17: 199Bv, 24-mer	5'-CCATCACCAGTTGCCACTATCTTT
all	non-sense	18: 200Bv, 15-mer	5'-CATGCCATCCGTAAG

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Fig. 3

Overview of all mutations

1(A-C)	
GGTGTGTTTAATTATGAGACTGAGACCACCTCTGTTATCCCAGCAGCTCGACTGTTCAAG	60
G V F N Y E T E T T-P S V I P A A R L F K	20
9(A-G) 2(A-C) 2(A-C)	
GCCTTTATCCTTGATGGCGATAACCTCTTTCCAAAGGTTGCACCCCAAGCCATTAGCAGT	120
A F I L D-G G D N-T L F P K-Q V A P Q A I S S	40
3(GA-TC) 7(AA-TG) 4(G-C) 6(GA-TC)	
GTTGAAAACATTGAAGGAAATGGAGGGCCTGGAACCATTAAAGAAGATCAGCTTTCCCGAA	180
V E N I E-S G N-S G G P G T I K K-N I S F P E-S	60
5(CA-TG)	
GGCCTCCCTTTCAAGTACGTGAAGGACAGAGTTGATGAGGTGGACCACACAAACTTCAAA	240
G L P F K Y V K D R V D E V D H T-A N F K	80
TACAATTACAGCGTGATCGAGGGCGGTCCCATAGGCGACACATTGGAGAAGATCTCCAAC	
Y N Y S V I E G G P I G D T L E K I S N	100
10(GAG-CAC) 8(CCC-TGG)	
GAGATAAAGATAGTGGCAACCCCTGATGGAGGATCCATCTTGAAGATCAGCAACAAGTAC	360
E I K I V A T P-G D G G S I L K I S N K Y	120
CACACCAAAGGTGACCATGAGGTGAAGGCAGAGCAGGTTAAGGCAAGTAAAGAAATGGGC	
H T K G D H E V K A E Q V K A S K E M G	140
GAGACACTTTTGAGGGCCGTTGAGAGCTACCTCTTGGCACACTCCGATGCCCTAGAACTAA	
E T L L R A V E S Y L L A H S D A Y N stop	159

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FIG. 4

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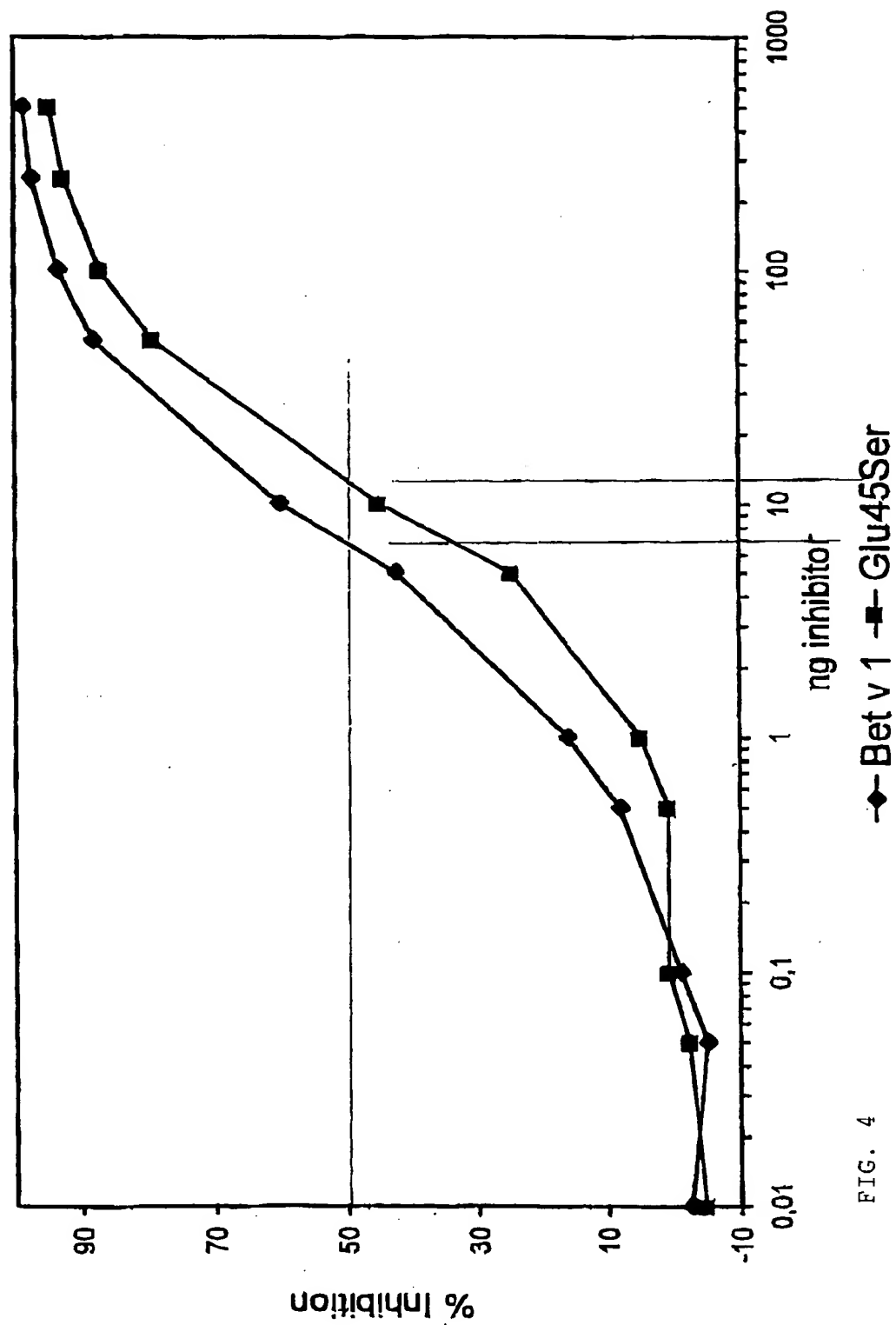


FIG. 4

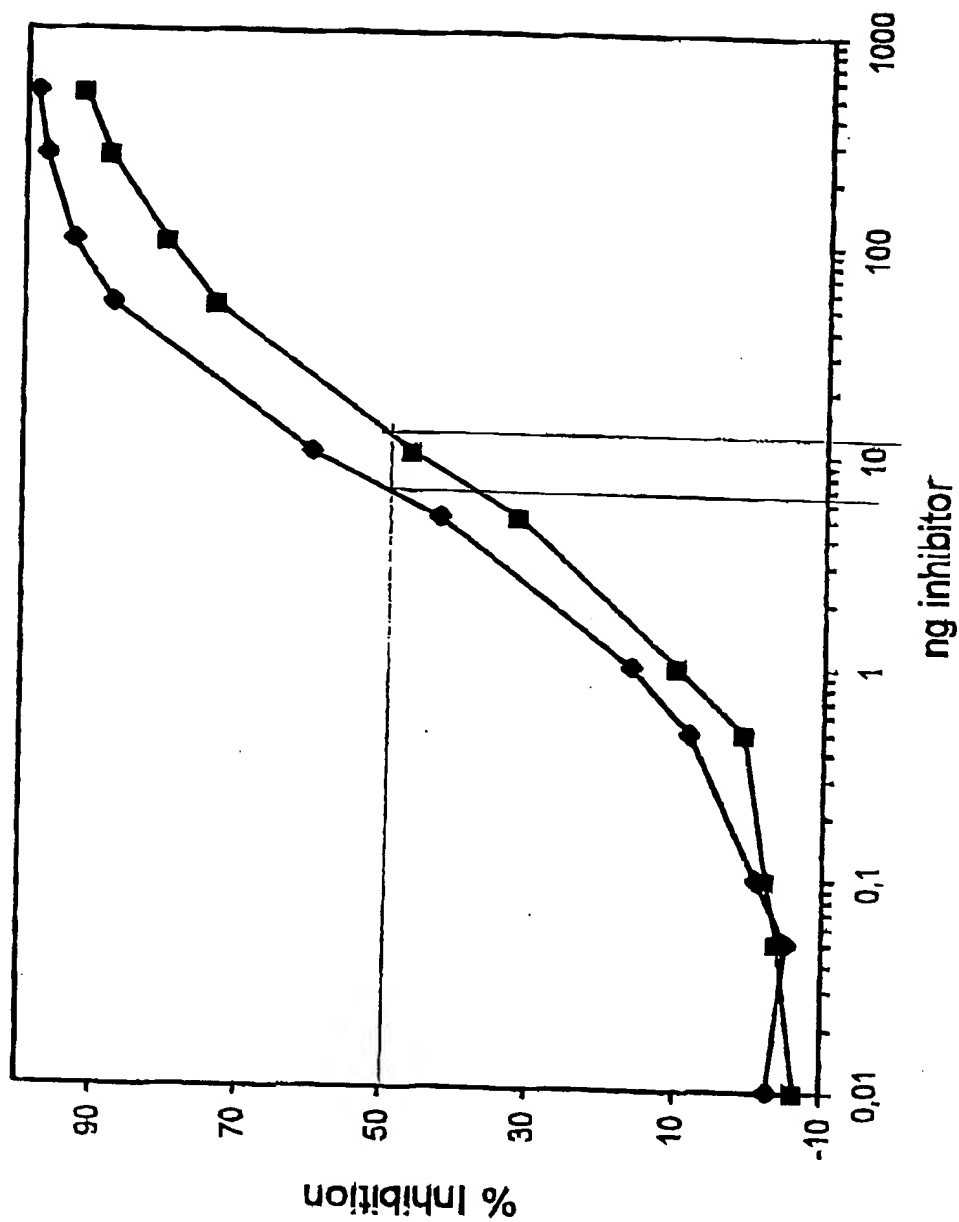
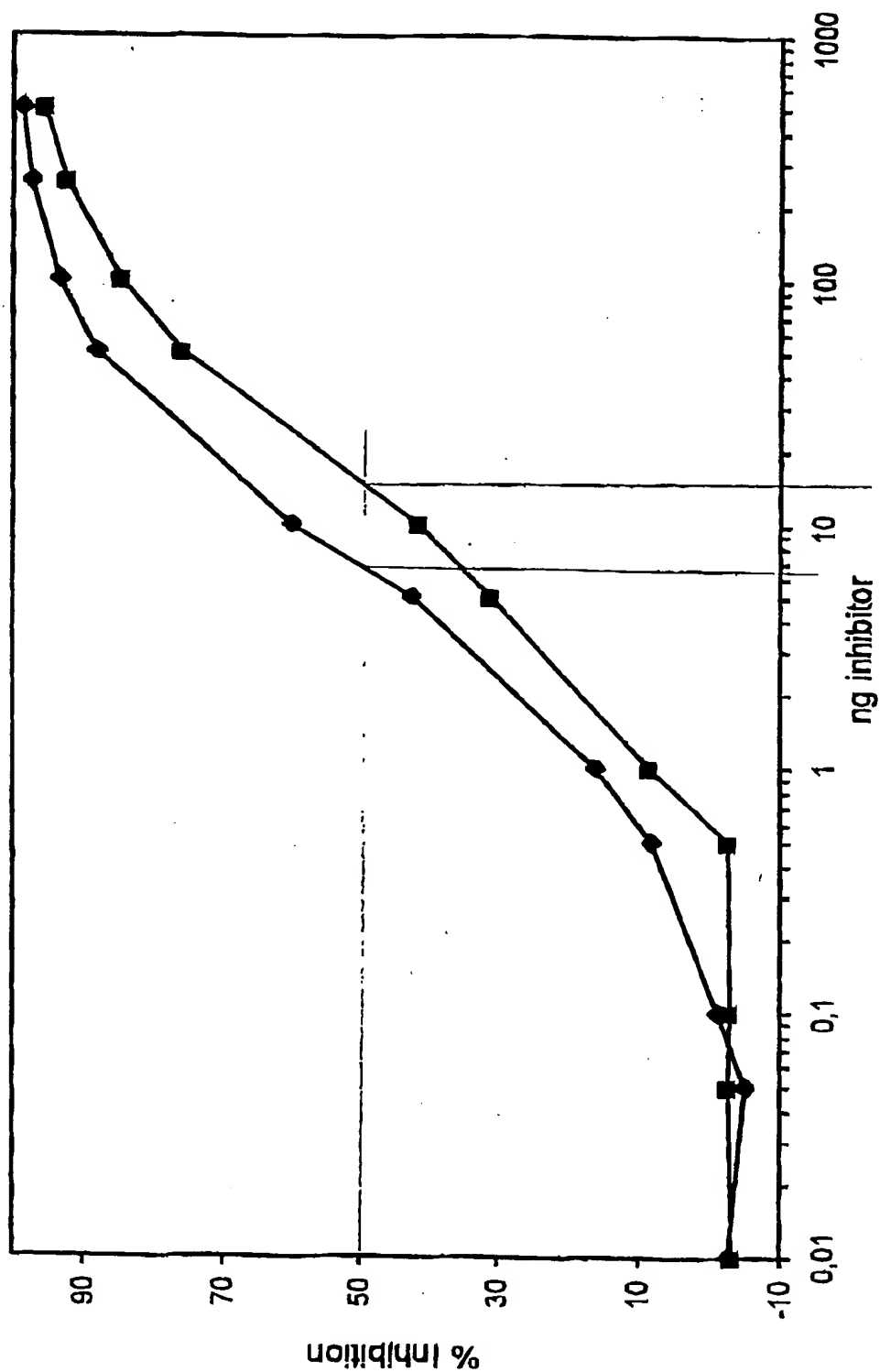


FIG. 5 — Bet v 1 — Asn28Thr+Lys32Gln

Fig. 6

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◆ Bet v 1 ■ Pro108Gly

FIG. 6

Fig 7

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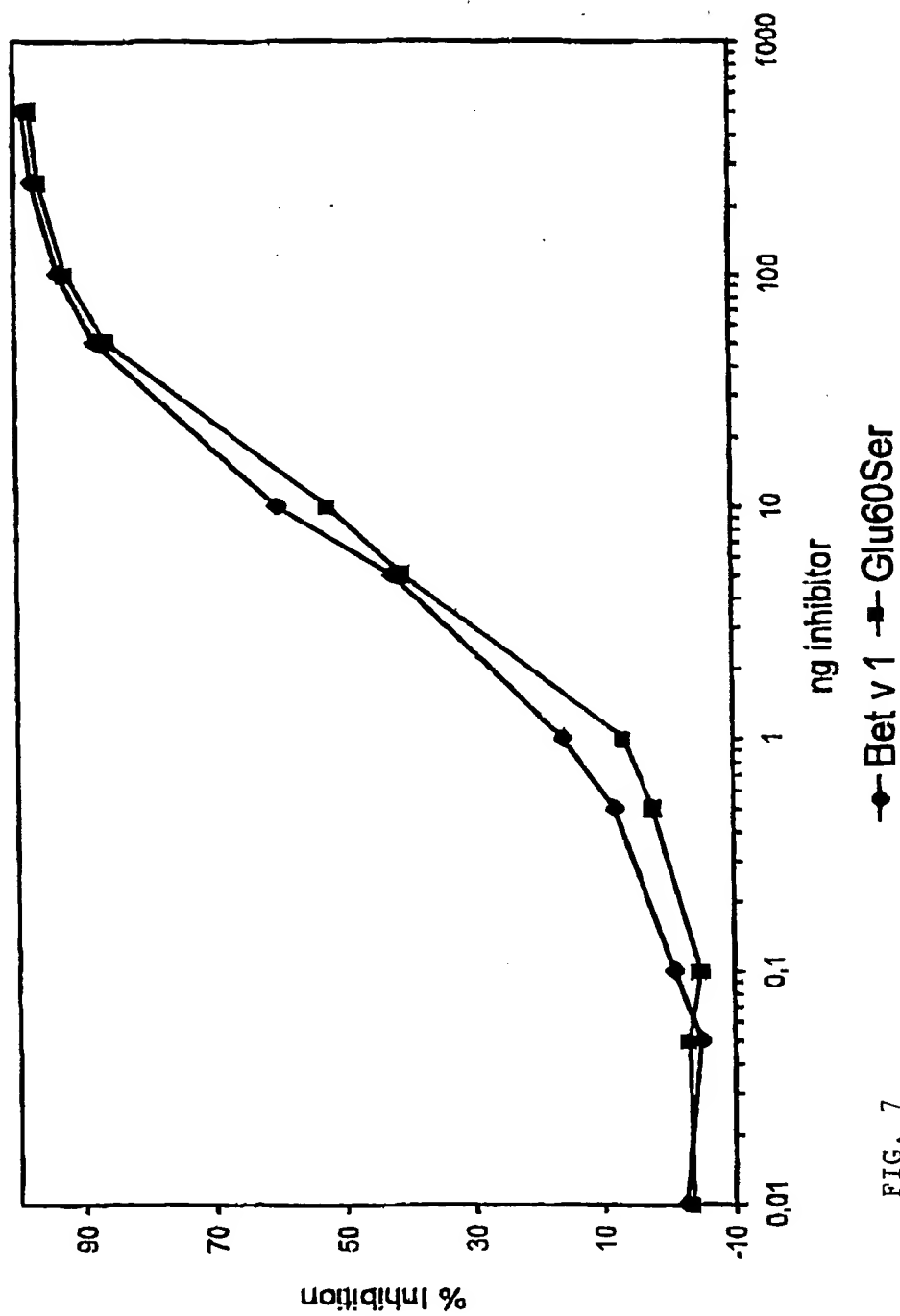
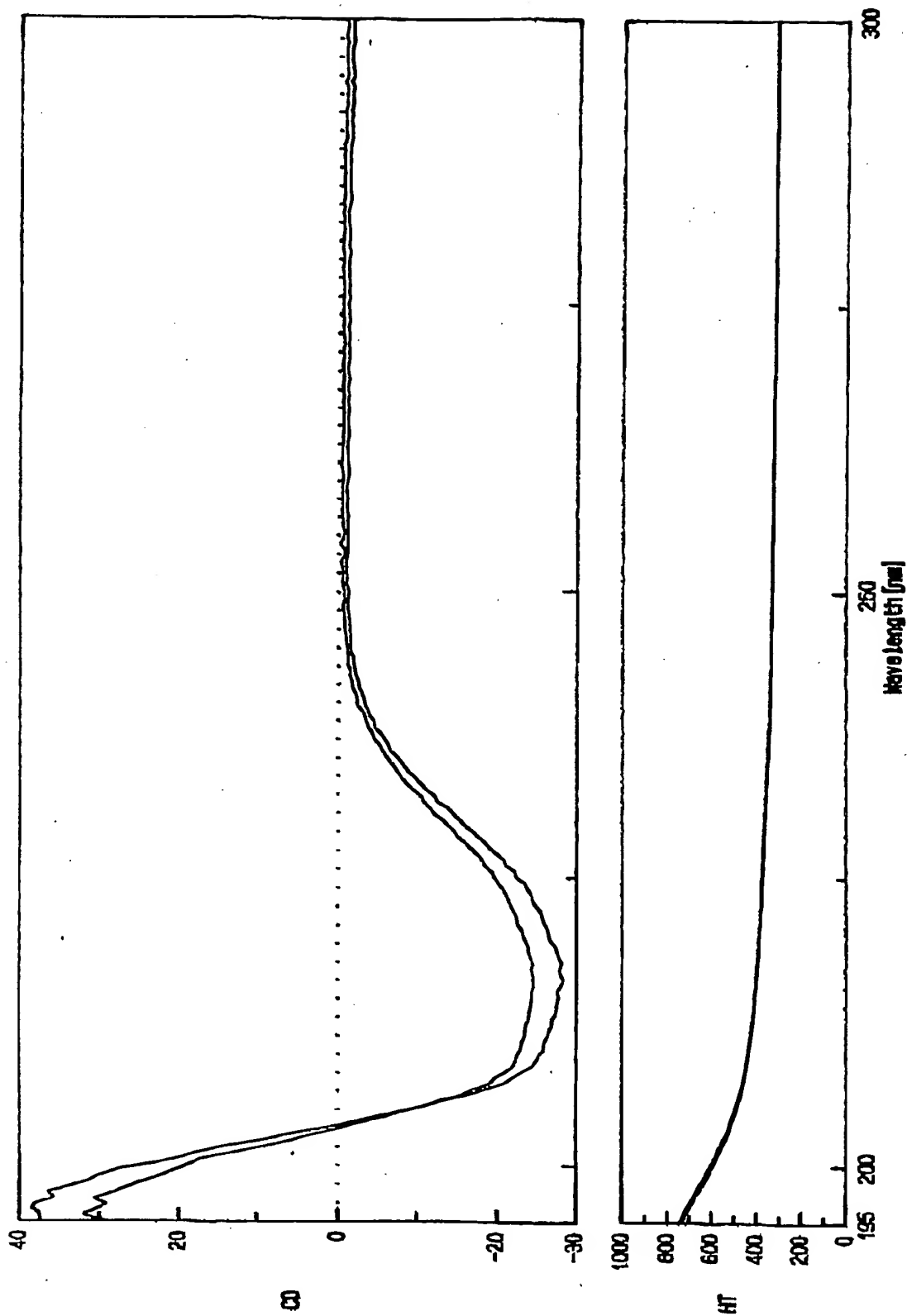


FIG. 7

Fig. 8

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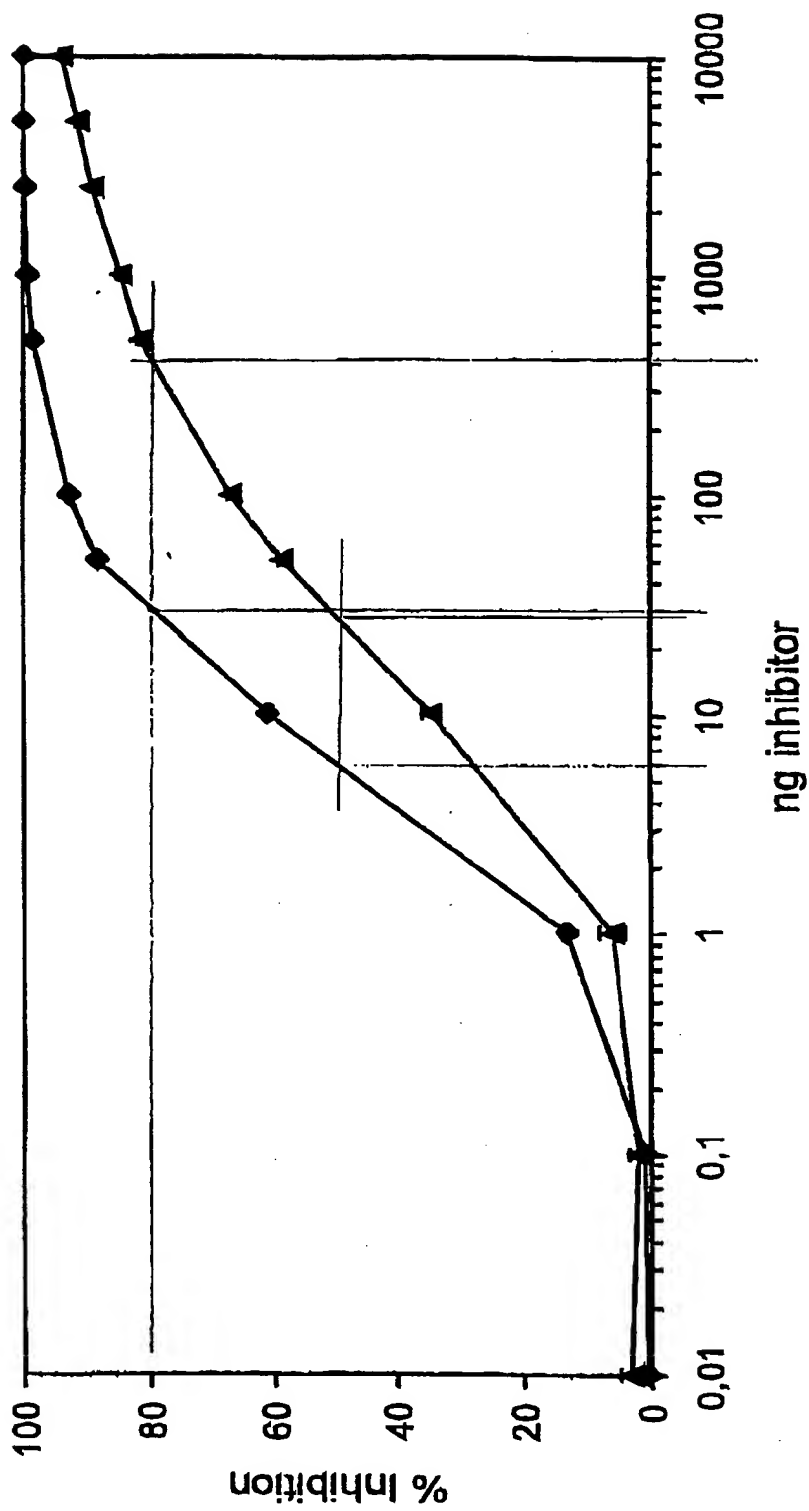


— 5798a r Betv 2801
 - - - 5797a 3#Mutant 2595

FIG. 8

Fig. 9

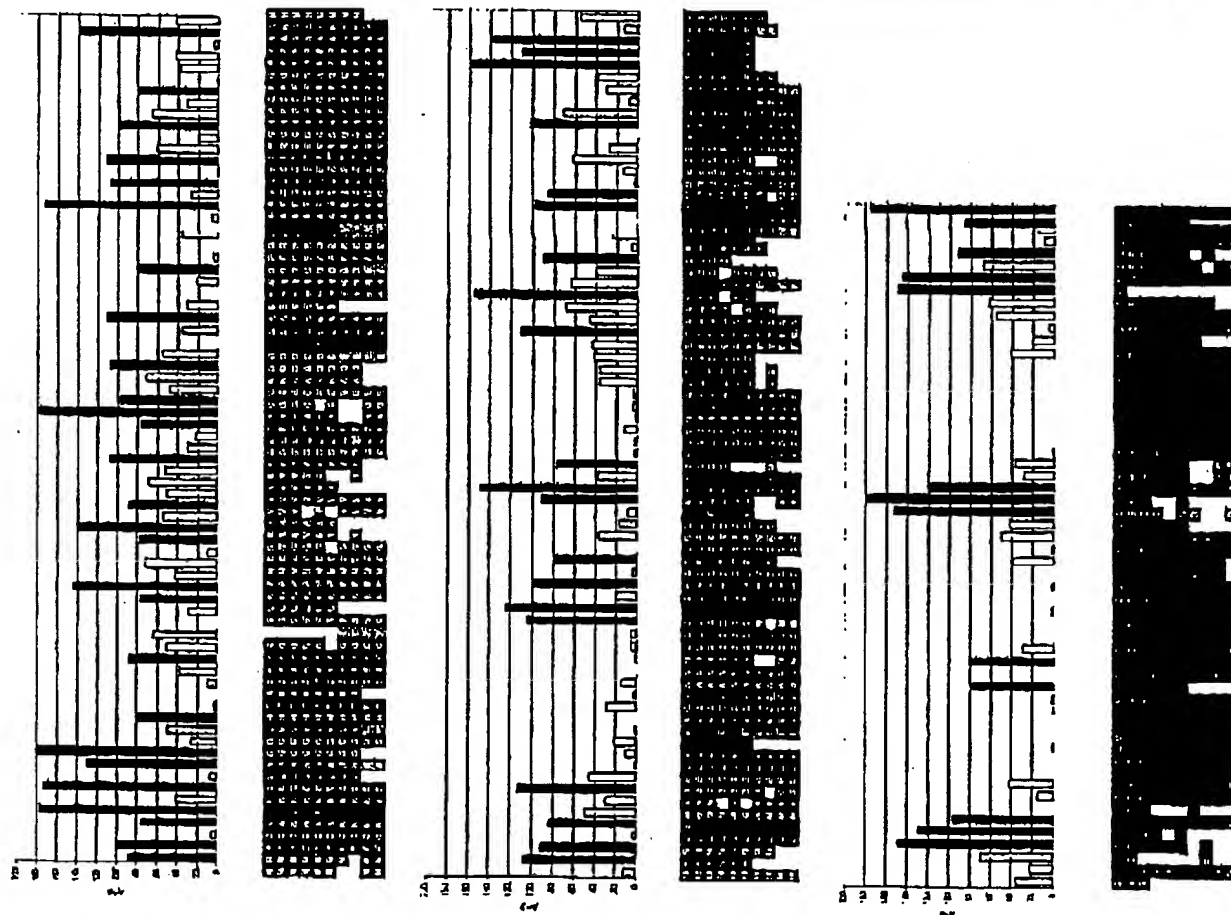
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◆ Bet v 1 —▲ Glu45Ser, Pro108Gly, Asn28Thr+Lys32Gln.

FIG. 9

Conserved residues among Vespula antigen 5



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FIG. 10

Fig.11

Mutant-specific oligonucleotide primers used for Ves v 5 mutants.
Mutated nucleotides underlined.

Ves v 5 mutant 1 (K72A)

Ves v 5 sense	5'-	ACCACAGCCTCCAGCGAAGAATATGAAAAATTGGTATGGA	-3'
Ves v 5 non-sense	3'-	TGGTGTGGGAGGTCGCTTCTTATACTTTTAAACCATACCT	-5'
sense primer	5'-	CCAGCG <u>CT</u> AATATGAAAAAT	-3'
non-sense primer	3'-	GTCGGAGGTCG <u>CG</u> ATTATAC	-5'

Ves v 5 mutant 2 (Y96A)

Ves v 5 sense	5'-	GGCTAATCAATGTCAATATGGTCACGATACTTGCAGGGATG	-3'
Ves v 5 non-sense	3'-	CCGATTAGTTACAGTTATACCAGTCTATGAACGTCCCTAC	-5'
sense primer	5'-	TGTCAAG <u>CT</u> GGTCACGATACT	-3'
non-sense primer	3'-	TTAGTTACAGTT <u>CG</u> ACCAGTG	-5'

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Fig. 12

Oligonucleotide primers for site directed mutagenesis of Ves v 5.

all sense 1: XhoI start, 38-mer:

EcoRI
 5'-CCGCTCGAGAAAGAAACAATTATTGTAAATATAAATG
 L E K R N N Y C K I K
 Kex2 cleavage site amino terminus of Ves v 5

1	sense	1: K72As	21-mer	5'-CCAGCGGCTAATATGAAAAAT
1	non-sense	2: K72Aa	21-mer	5'-CATATTAGCCGCTGGAGGCTG
2	sense	3: Y96As	21-mer	5'-TGTCAAGCTGGTCACGATACT
2	non-sense	4: Y96Aa	21-mer	5'-GTGACCAGCTTGACATTGATT
all non-sense 7: CT-pPICZαA, 21-mer				5'-ATTTCATCAGCTGCGAGATAGG

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Fig. 13

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Overview of Ves v 5 mutations

1	AACAATTATTGTAAAATAAAATGTTTGAAAGGAGGTGTCCATACTGCCTGCAAATATGGA	60
1	N N Y C K I K C L K G G V H T A C K Y G	20
61	AGTCTTAAACCGAATTGCGGTAATAAGGTAGTGGTATCCTATGGTCTAACGAAACAAGAG	120
21	S L K P N C G N K V V V S Y G L T K Q E	40
121	AAACAAGACATCTTAAAGGAGCACAATGACTTTAGACAAAAAATTGCACGAGGATTGGAG	180
41	K Q D I L K E H N D F R Q K I A R G L E	60
	1 [K72A] (AAG-GCT)	
181	ACTAGAGGTAATCCTGGACCACAGCCTCCAGCGAAGAAATATGAAAAATTTGGTATGGAAC	240
61	T R G N P G P Q P P A K N M K N L V W N	80
	2 [Y96A] (TA-GC)	
241	GACGAGTTAGCTTATGTGCGCCCAAGTGTGGGCTAATCAATGTCAATATGCTCAGGATACT	300
81	D E L A Y V A Q V W A N Q C Q Y G H D T	100
301	TGCAGGGATGTAGCAAAATATCAGGTTGGACAAAACGTAGCCTTAACAGGTAGCACGGCT	360
101	C R D V A K Y Q V G Q N V A L T G S T A	120
361	GCTAAATACGATGATCCAGTTAAACTAGTTAAATGTGGGAAGATGAAGTGAAAGATTAT	420
121	A K Y D D P V K L V K M W E D E V K D Y	140
421	AATCCTAAGAAAAAGTTTTCGGGAAACGACTTTCTGAAAACCGGCCATTACACTCAAATG	480
141	N P K K K F S G N D F L K T G H Y T Q M	160
481	GTITGGGCTAACACCAAGGAAGTTGGTTGTGGAAGTATAAAATACATTCAAGAGAAATGG	540
161	V W A N T K E V G C G S I K Y I Q E K W	180
541	CACAAACATTACCTTGTATGTAATTATGGACCCAGCGGAAACTTTAAGAATGAGGAACTT	600
181	H K H Y L V C N Y G P S G N F K N E E L	200
601	TATCAAAACAAAGTAA	612
201	Y Q T K stop	204

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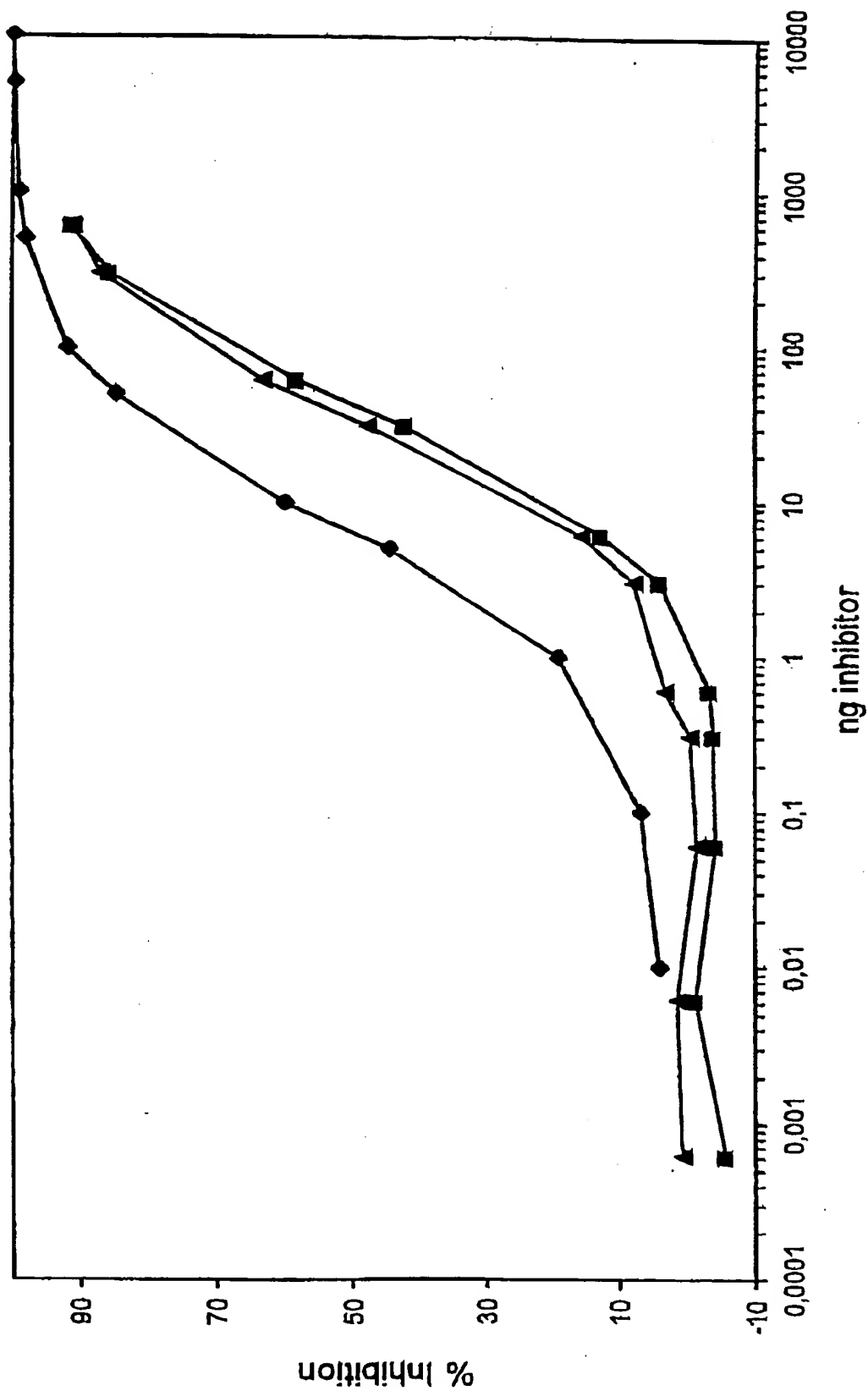


FIG. 14